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(54) Title: FUMONOSIN RESISTANCE

(57) Abstract

DNA encoding a protein capable of increasing resistance to mycotoxins of the fumonosin family is described, as are gene transfer vectors useful for imparting fumonosin resistance (e.g., resistance to fumonosin B1) to a plant or animal. The vector comprises an expression cassette, the expression cassette contains a DNA encoding a fumonosin–resistance protein. Methods of making fumonosin–resistant transgenic plants and animals, and fumonosin–resistant transgenic plants and animals, are also described.

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FUMONOSIN RESISTANCE

This application claims the benefit of U.S. Provisional Application No. 60/057,562; filed 26 August 1997.

This invention was made with Government support under grant number IR29-AG-12467 from the National Institutes of Health. The Government has certain rights to this invention.

Field of the Invention

The present invention relates to DNA encoding proteins that increase resistance to the mycotoxin fumonosin in plant and animal cells, and to transgenic plants and animals having increased resistance to mycotoxins of the fumonosin family, such as fumonosin B1.

Background of the Invention

The fumonosins are a family of mycotoxins that are common contaminants of maize, sorghum and related grains throughout the world. These compounds were first identified in a study of a high incidence of oesophageal cancer in certain villagers in the Transkei region of South Africa. The villagers consumed beer brewed from moldy corn infected by *Fusarium moniliforme*, which produces fumonosin B1. A. Merrill et al., *Trends in Cell Biology* **6**, 218 (June 1996).

Fumonosin B1, the most common of the fumonosins, is produced by *F. moniliforme* (Sheldon). The toxin is also implicated in two devastating and costly diseases of veterinary animals: equine leukoencephalomacin and porcine pulmonary oedema. *Id.* Fumonosin B1 is both toxic and carcinogenic to plants and animals.

In view of the foregoing, it would be extremely useful to have a means for imparting fumonosin resistance to plants, particularly grains and other monocots, as well as plants and animals susceptible to infection with a fumonosin-producing fungi.

Summary of the Invention

A first aspect of the present invention is a plant or animal gene transfer vector useful for imparting fumonosin resistance to a plant or animal. The vector comprising an expression cassette, the expression cassette contains a DNA encoding a fumonosin-resistance protein (*e.g.*, an ATP-binding cassette transporter). In general, such a DNA is *(a)* a DNA having a sequence according to **SEQ ID NO:1**, *(b)* a DNA that hybridizes to DNA having a sequence according to **SEQ ID NO:1** and encodes and an ATP-binding cassette transporter that imparts fumonosin-resistance to a plant or animal cell, or *(c)* a DNA that encodes a protein encoded by a DNA of (a) or (b) above, but differs from the DNA of (a) or (b) above due to the degeneracy of the genetic code.

A second aspect of the present invention is a method of making a fumonosin-resistant transgenic plant. The method comprises transforming a plant cell with an expression cassette as described above, and then regenerating a fumonosin-resistant transgenic plant from the transformed plant cell.

A third aspect of the present invention is a fumonosin-resistant transgenic plant, wherein some or all of the cells of the plant contain a heterologous expression cassette as described above.

A fourth aspect of the present invention is a method of making a fumonosin-resistant transgenic animal. The method comprises transforming an

animal cell with an expression cassette as described above, and then regenerating a fumonosin-resistant transgenic animal from the transformed animal cell.

A fifth aspect of the present invention is a fumonosin-resistant transgenic non-human animal, wherein some or all of the cells of the animal containing a heterologous expression cassette as described above.

The foregoing and other objects and aspects of the present invention are explained in greater detail in the specification set forth below.

Detailed Description of the Invention

The present invention may be used to impart resistance to any type of fumonosin to plants and animals, including fumonosins of the A, B, and C series (*e.g.*, fumonosin A1, fumonosin B1, fumonosin B2, fumonsin C1, phytotoxin TA). The imparting of resistance to B series fumonosins is preferred, and the imparting of resistance to fumonosin B1 is most preferred. The term "resistance" as used herein does not imply complete resistance, but rather refers to any increase in the level of resistance that is of a commercial agriculatural or veterinary advantage as compared to the same animal without the presence of the expression cassette.

A. DNA sequences

DNAs sequences useful for carrying out the present invention include those coding for ATP-binding cassette (ABC) transporters, and particularly for proteins homologous to, and having essentially the same biological properties as, the protein given herein SEQ ID NO:2. This definition is intended to encompass natural allelic variations therein. Isolated DNA or cloned genes of the present invention can be of any species of origin, including microorganism, plant, and animal, (*see generally* C. Higgins, ABC Transporters: from microorganisms to man, *Annu. Rev. Cell Biol. 8:* 67 (1992)), but are typically of natural origin and are preferably of yeast origin. Thus, DNAs which hybridize to DNA disclosed herein as SEQ ID NO:1 (or fragments or derivatives thereof which serve as hybridization probes as discussed below) and which code on expression for a protein of the present

invention (e.g., a protein according to SEQ ID NO:2) are also an aspect of this invention.

Conditions which will permit other DNAs which code on expression for a protein of the present invention to hybridize to the DNA of SEQ ID NO:1 disclosed herein can be determined in accordance with known techniques. For example, hybridization of such sequences may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% Formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to DNA of SEQ ID NO:1 disclosed herein in a standard hybridization assay. *See, e.g.*, J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, sequences which code for proteins of the present invention and which hybridize to the DNA of SEQ ID NO:1 disclosed herein will be at least 75% homologous, 85% homologous, and even 95% homologous or more with SEQ ID NO:1.

DNAs which code for proteins of the present invention, or DNAs which hybridize to that of SEQ ID NO:1, but which differ in codon sequence from SEQ ID NO:1 due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the literature. *See, e.g.*, U.S. Patent No. 4,757,006 to Toole et al. at Col. 2, Table 1.

Knowledge of the nucleotide sequence as disclosed herein in SEQ ID NO:1 can be used to generate hybridization probes which specifically bind to the DNA of the present invention or to mRNA to determine the presence of amplification or overexpression of the proteins of the present invention. Pairs of probes which will serve as PCR primers for the DNA sequences of the present invention, or portions thereof, may be used in accordance with the process described in U.S. Patents Nos. 4,683,202 and 4,683,195 to Mullis (applicant specifically intends that the

disclosures of all U.S. Patent references disclosed herein be incorporated herein by reference).

Since numerous ATP-binding cassette (ABC) transporters are known, *see, e.g.*, C. Higgins, *Annu. Rev. Cell Biol.* 8, 67 (1992), ABC transporters that impart fumonosin resistance to plant or animal cells when expressed therein can also be identified by expressing that transporter in a plant or animal cell, or a yeast cell, and then testing that cell for fumonosin resistance, essentially as described below.

B. Genetic Engineering Techniques

The production of cloned genes, recombinant DNA, vectors, transformed host cells, proteins and protein fragments by genetic engineering is well known. *See, e.g.*, U.S. Patent No. 4,761,371 to Bell et al. at Col. 6 line 3 to Col. 9 line 65; U.S. Patent No. 4,877,729 to Clark et al. at Col. 4 line 38 to Col. 7 line 6; U.S. Patent No. 4,912,038 to Schilling at Col. 3 line 26 to Col. 14 line 12; and U.S. Patent No. 4,879,224 to Wallner at Col. 6 line 8 to Col. 8 line 59. (Applicant specifically intends that the disclosure of all U.S. patent references cited herein be incorporated by reference herein in their entirety).

DNA constructs of the present invention may be used to transform cells from a variety of organisms, including plants (*i.e.*, vascular plants) and animals (particularly mammals such as horses, cows and pigs). As used herein, plants includes both gymnosperms and angiosperms (*i.e.*, monocots and dicots). Transformation according to the present invention may be used to increase expression levels of transgenes in stably transformed cells.

The term "operatively associated," as used herein, refers to DNA sequences on a single DNA molecule which are associated so that the function of one is affected by the other. Thus, a transcription initiation region is operatively associated with a structural gene when it is capable of affecting the expression of that structural gene (i.e., the structural gene is under the transcriptional control of the transcription initiation region). The transcription initiation region is said to be "upstream" from the structural gene, which is in turn said to be "downstream" from the transcription initiation region.

DNA constructs, or "expression cassettes," of the present invention preferably include, 5' to 3' in the direction of transcription, a transcription initiation region, a structural gene operatively associated with the transcription initiation region, and a termination sequence including a stop signal for RNA polymerase and a polyadenylation signal for polyadenylation (e.g., the nos terminator. All of these regions should be capable of operating in the cells to be transformed. Matrix attachment regions flanking the expression cassette may optionally be included. The termination region may be derived from the same gene as the transcription initiation or promoter region, or may be derived from a different gene.

The transcription initiation region, which preferably includes the RNA polymerase binding site (promoter), may be native to the host organism to be transformed or may be derived from an alternative source, where the region is functional in the host. Other sources include the Agrobacterium T-DNA genes, such as the transcriptional initiation regions for the biosynthesis of nopaline, octapine, mannopine, or other opine transcriptional initiation regions, transcriptional initiation regions from plants, transcriptional initiation regions from viruses (including host specific viruses), or partially or wholly synthetic transcription initiation regions. Transcriptional initiation and termination regions are well known. *See, e.g.,* dGreve, *J. Mol. Appl. Genet.* 1, 499-511 (1983); Salomon et al., *EMBO J.* 3, 141-146 (1984); Garfinkel et al., *Cell* 27, 143-153 (1983); and Barker et al., *Plant Mol. Biol.* 2, 235-350 (1983).

The transcriptional initiation regions may, in addition to the RNA polymerase binding site, include regions which regulate transcription, where the regulation involves, for example, chemical or physical repression or induction (e.g., regulation based on metabolites or light) or regulation based on cell differentiation (such as associated with leaves, roots, seed, or the like in plants). Thus, the transcriptional initiation region, or the regulatory portion of such region, is obtained from an appropriate gene which is so regulated. For example, the 1,5-ribulose biphosphate carboxylase gene is light-induced and may be used for transcriptional initiation. Other genes are known which are induced by stress, temperature, wounding, pathogen effects, etc. Tissue specific promoters, such as

root-specific promoters or a promoter specific for corn silk, may advantageously be employed as will be apparent to those skilled in the art. In corn for the prevention of corn ear rot (or "pink ear rot of maize"), a promoter associated with Pedicel Glutamine Synthetase gene (preferentially expressed in the region of the kernel attached to the cob, where fumonosin-fungi enter the kernel) may be employed, or a promoter that preferentially expresses in the seed or seed coat.

The expression cassette may be provided in a DNA construct that also has at least one replication system. For convenience, it is common to have a replication system functional in *Escherichia coli*, such as ColE1, pSC101, pACYC184, or the like. In this manner, at each stage after each manipulation, the resulting construct may be cloned, sequenced, and the correctness of the manipulation determined. In addition, or in place of the *E. coli* replication system, a broad host range replication system may be employed, such as the replication systems of the P-1 incompatibility plasmids, e.g., pRK290. In addition to the replication system, there will frequently be at least one marker present, which may be useful in one or more hosts, or different markers for individual hosts. That is, one marker may be employed for selection in a prokaryotic host, while another marker may be employed for selection in a eukaryotic host, particularly a plant host. The markers may be protection against a biocide, such as antibiotics, toxins, heavy metals, or the like; provide complementation, for example by imparting prototrophy to an auxotrophic host; or provide a visible phenotype through the production of a novel compound. Exemplary genes that may be employed include neomycin phosphotransferase (NPTII), hygromycin phosphotransferase (HPT), chloramphenicol acetyltransferase (CAT), nitrilase, and the gentamicin resistance gene. For plant host selection, non-limiting examples of suitable markers are β-glucuronidase, providing indigo production, luciferase, providing visible light production, NPTII, providing kanamycin resistance or G418 resistance, HPT, providing hygromycin resistance, and the mutated aroA gene, providing glyphosate resistance.

The various fragments comprising the various constructs, expression cassettes, markers, and the like may be introduced consecutively by restriction enzyme cleavage of an appropriate replication system, and insertion of the

particular construct or fragment into the available site. After ligation and cloning the DNA construct may be isolated for further manipulation. All of these techniques are amply exemplified in the literature and find particular exemplification in Sambrook et al., Molecular Cloning: A Laboratory Manual, (2d Ed. 1989)(Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

C. Plant Genetic Engineering

As noted above, the present invention provides a method of making a fumonosin-resistant transgenic plant. The term "plant" as used herein refers to vascular plants (*e.g.*, gymnosperms and angiosperms). The method comprises transforming a plant cell with an expression cassette as described above, and then regenerating a fumonosin-resistant transgenic plant from the transformed plant cell. The transforming step may be carried out by any suitable means, including by *Agrobacterium*-mediated transformation and non-*Agrobacterium*-mediated transformation, as discussed in detail below. Plants are regenerated from the transformed cell (or cells) by techniques known to those skilled in the art, as also discussed below. Where chimeric plants are produced by the process, plants in which all cells are transformed may be regenerated from chimeric plants having transformed germ cells, as is known in the art.

Vectors that may be used to transform plant tissue with DNA constructs/expression cassettes of the present invention include both *Agrobacterium* and non-*Agrobacterium* vectors, particularly ballistic vectors, as well as vectors suitable for DNA-mediated transformation.

***Agrobacterium* mediated transformation.** *Agrobacterium*-mediated gene transfer exploits the natural ability of *Agrobacterium tumefaciens* to transfer DNA into plant chromosomes. *Agrobacterium* is a plant pathogen that transfers a set of genes encoded in a region called T-DNA of the Ti plasmid into plant cells at wound sites. The typical result of gene transfer is a tumorous growth called a crown gall in which the T-DNA is stably integrated into a host chromosome. The ability to cause crown gall disease can be removed by deletion of the genes in the T-DNA without loss of DNA transfer and integration. The DNA to be

transferred is attached to border sequences that define the end points of an integrated T-DNA.

The *Agrobacterium* strain utilized in the methods of the present invention is modified to contain a gene or genes of interest, or a nucleic acid to be expressed in the transformed cells. The nucleic acid to be transferred is incorporated into the T-region and is flanked by T-DNA border sequences. A variety of *Agrobacterium* species are known in the art particularly for dicotyledon transformation. Such *Agrobacterium* can be used in the methods of the invention. *See, e.g.*, Hooykaas, *Plant Mol. Biol.* 13, 327 (1989); Smith et al., *Crop Science* 35, 301 (1995); Chilton, *Proc. Natl. Acad. Sci. USA* 90, 3119 (1993); Mollony et al., *Monograph Theor. Appl. Genet NY* 19, 148 (1993); Ishida et al., *Nature Biotechnol.* 14, 745 (1996); and Komari et al., *The Plant Journal* 10, 165 (1996), the disclosures of which are incorporated herein by reference.

In addition to the T-region, the Ti plasmid contains a *vir* region. The *vir* region is important for efficient transformation, and appears to be species-specific. Binary vector systems have been developed where the manipulated disarmed T-DNA carrying foreign DNA and the *vir* functions are present on separate plasmids. In this manner, a modified T-DNA region comprising foreign DNA (the nucleic acid to be transferred) is constructed in a small plasmid which replicates in *E. coli*. This plasmid is transferred conjugatively in a tri-parental mating into *Agrobacterium tumefaciens* that contains a compatible plasmid-carrying virulence gene. The *vir* functions are supplied in *trans* to transfer the T-DNA into the plant genome. Such binary vectors are useful in the practice of the present invention.

Preferred vectors of the present invention are super-binary vectors. *See, e.g.*, United States Patent No. 5,591,615 and EP 0 604 662. Such a super-binary vector has been constructed containing a DNA region originating from the virulence region of the Ti plasmid pTiBo542 (Jin et al., *J. Bacteriol.* 169, 4417 (1987)) contained in a super-virulent *Agrobacterium tumefaciens* A281 exhibiting extremely high transformation efficiency (Hood et al., *Biotechnol.* 2, 702 (1984);

Hood et al., *J. Bacteriol.* **168**, 1283 (1986); Komari et al., *J. Bacteriol.* **166**, 88 (1986); Jin et al., *J. Bacteriol.* **169**, 4417 (1987); Komari, *Plant Science* **60**, 223 (1987); ATCC Accession No. 37394. Exemplary super-binary vectors known to those skilled in the art include pTOK162 (Japanese patent Appl. (Kokai) No. 4-222527, EP 504,869, EP 604,662, and United States Patent No. 5,591,616, herein incorporated by reference) and pTOK233 (Komari, *Plant Cell Reports* 9,303 (1990); Ishida et al., *Nature Biotechnology* **14**, 745 (1996); herein incorporated by reference). Other super-binary vectors may be constructed by the methods set forth in the above references. Super-binary vector pTOK162 is capable of replication in both *E. coli* and in *A. tumefaciens*. Additionally, the vector contains the *vir*B, *vir*C and *virG* genes from the virulence region of pTiBo542. The plasmid also contains an antibiotic resistance gene, a selectable marker gene, and the nucleic acid of interest to be transformed into the plant. The nucleic acid to be inserted into the sorghum genome is located between the two border sequences of the T region. Super-binary vectors of the invention can be constructed having the features described above for pTOK162. The T-region of the super-binary vectors and other vectors for use in the invention are constructed to have restriction sites for the insertion of the genes to be delivered. Alternatively, the DNA to be transformed can be inserted in the T-DNA region of the vector by utilizing *in vivo* homologous recombination. *See*, Herrera-Esterella et al., *EMBO J.* **2**, 987 (1983); Horch et al., *Science* **223**, 496 (1984). Such homologous recombination relies on the fact that the super-binary vector has a region homologous with a region of pBR322 or other similar plasmids. Thus, when the two plasmids are brought together, a desired gene is inserted into the super-binary vector by genetic recombination via the homologous regions.

Non-*Agrobacterium* mediated transformation. Microparticles carrying a DNA construct of the present invention, which microparticles are suitable for the ballistic transformation of a cell, are also useful as a vector for transforming cells according to the present invention. The microparticle is propelled into a cell to produce a transformed cell. Where the transformed cell is a plant cell, a plant may be regenerated from the transformed cell according to techniques known in the art.

Any suitable ballistic cell transformation methodology and apparatus can be used in practicing the present invention. Exemplary apparatus and procedures are disclosed in Sanford and Wolf, U.S. Patent No. 4,945,050. When using ballistic transformation procedures, the expression cassette may be incorporated into a plasmid capable of replicating in the cell to be transformed. Examples of microparticles suitable for use in such systems include 1 to 5 μm gold spheres. The DNA construct may be deposited on the microparticle by any suitable technique, such as by precipitation.

Plant species may be transformed with the DNA construct of the present invention by the DNA-mediated transformation of plant cell protoplasts and subsequent regeneration of the plant from the transformed protoplasts in accordance with procedures well known in the art.

Any plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a vector of the present invention. The term "organogenesis," as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers; the term "embryogenesis," as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

Plants of the present invention may take a variety of forms. The plants may be chimeras of transformed cells and non-transformed cells; the plants may be clonal transformants (e.g., all cells transformed to contain the expression cassette); the plants may comprise grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). The transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, first generation (or T1)

transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques. A dominant selectable marker (such as *npt* II) can be associated with the expression cassette to assist in breeding.

Plants that may be employed in practicing the present invention include (but are not limited to) maize or corn (*Zea mays*), sorghum, wheat, oats, rye, barley, rice, tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), soybean (*glycine max*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Cofea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), vegetables, ornamentals, and conifers.

Particularly preferred plants for carrying out the present invention are maize (*Zea mays*) and sorghum.

D. Transgenic Animals.

A method of making a fumonosin-resistant transgenic animal is also an aspect of the present invention. The method can be carried out on any suitable animal subject, but is preferably carried out with non-human mammals. Ovine, bovine, and equine species are particularly preferred (e.g., pigs, cows, and horses).

The method comprises transforming an animal cell with an expression cassette as described above, in an animal transformation vector, and then regenerating a fumonosin-resistant transgenic animal from the transformed animal cell. The transformation step may be carried out by any suitable means, as discussed in detail below, and the regeneration step may also be carried out by any suitable means, as also discussed in detail below. Where chimeric animals are produced by the process, animals in which all cells are transformed may be

regenerated from chimeric animals having transformed germ cells, as is known in the art.

The production of transgenic animals can be carried out by any suitable technique, such as pronuclear microinjection, infection of embryos with retroviruses, embryonic stem cell-mediated techniques, transfer of entire chromosomal segments and gamete transfection in conjunction with *in vitro* fertilization, etc. *See generally* Charles River Laboratories, *Transgenic Animal Science: Principles and Methods* (Summer 1991).

Transgenic animals that express an ABC transporter protein can be produced by the genetic transformation of zygotes, as described in T. Wagner et al., U.S. Patent No. 4,873,191 (applicant intends that the disclosure of all U.S. Patent References cited herein be incorporated herein by reference).

Methods of producing a transgenic bovine or transgenic bovine embryo are described in U.S. Patent No. 5,663,076 to H. DeBoer et al.

In another technique, a pluripotent embryonic stem cell from the species to be transformed may be derived, the expression cassette inserted into the stem cell, and one or more of the stem cells inserted into an early embryo such as a blastocyst of the animal to be transformed, and the animal raised to birth in a suitable female host (*e.g.*, M. Evans, PCT Application WO90/03432).

In still another technique, embryonic stem cells useful for making chimeric and transgenic ungulates (e.g., porcine, bovine, ovine and caprine species) are described in M. Wheeler, PCT Application WO 94/26884. In general, the embryonic stem cells are transformed with the exogenous genetic material of interest (e.g., an ABC transporter expression cassette) and then used to provide chimeric ungulates which have germ cells comprising the exogenous genetic material. The chimeric ungulates are bred to provide transgenic ungulates (see also U.S. Patent No. 5,523,226 to M. Wheeler).

Methods of producing transgenic animals by subjecting a mixture of DNA and the embryo to an electric discharge are described in U.S. Patent No. 5,567,607 to X. Zhao et al.

Mammalian expression vectors are described in U.S. Patent No. 5,627,033 to J. Smith et al.

E. Utilities

The methods, constructs, and products described above are useful in providing a selectable marker (*i.e.*, Fumonosin resistance) for genetic engineering techniques, where other nucleic acid segments are being introduced into the plant or animal cell in association with the fumonosin-resistance gene.

The methods, constructs, and products described above are useful in providing plants and animals that are resistant, or have greater levels of resistance, to naturally occuring fumonosin infection.

The examples that follow are provided to illustrate the present invention, and are not to be construed as limiting thereof.

EXPERIMENTAL

A Fumonosin B1 sensitive *Sacharomyces cerevisiae* strain (JS16) was screened, which was sensitive to 400 μM Fumonosin B1 in synthetic complete medium at a cell density as high as 10^7 cells/mL (Wild-type yeast strain JK93da is resistant to as high as 1 mM Fumonosin B1). Then, a *S. cerivisiae* genomic DNA library was constructed in the multicopy vector YEP24. JS16 was transformed with the DNA library and four Fumonosin B1 resistant clones were selected on synthetic complete agar medium with 400 μM Fumonosin B1.

DNA sequencing was carried out among the four clones. Two of them had the same DNA sequence, the other two had the consensus DNA sequences as the first two clones. The consensus sequence (SEQ ID NO:1) encodes a protein of 1477 amino acid residues (SEQ ID NO:2). Overexpression of the protein confers Fumonosin B1 resistance to *S. cerivasiae*.

The gene deletion mutant is sensitive to Fumonosin B1 compared to wild type strain (JK93da).

A yeast database was searched. It was found that the protein belongs to an ABC transport family. *See* D. Katzmann et al., *Molecular and Cellular Biology* 15, 6875 (1995). The gene is located on chromosome VII.

That which is claimed is:

1. A DNA construct comprising an expression cassette, which construct comprises, in the 5' to 3' direction, a promoter operable in a plant cell and a DNA segment according to claim 1 positioned downstream from said promoter and operatively associated therewith, said DNA segment selected from the group consisting of:

(a) SEQ ID NO:1;

(b) DNA sequences which encode an enzyme having SEQ ID NO:2;

(c) DNA sequences which hybridize to isolated DNA of (a) or (b) above and which encode an ATP-binding cassette transporter; and

(d) DNA sequences which differ from the DNA of (a), (b) or (c) above due to the degeneracy of the genetic code.

2. A DNA construct according to claim 1, wherein said promoter is constitutively active in plant cells.

3. A DNA construct according to claim 1, wherein said promoter is selectively active in plant tissue cells.

4. A DNA construct according to claim 3, wherein said promoter is selectively active in plant cells selected from root cells, seed cells, seed coat cells, and corn silk cells.

5. A DNA construct according to claim 3, wherein said promoter is a corn Pedicel Glutamine Synthetase promoter that is expressed in the region of the corn kernel attached to the cob.

6. A DNA construct according to claim 1, wherein said construct further comprises a plasmid.

7. A DNA construct according to claim 1 carried by a plant transformation vector.

8. A DNA construct according to claim 1 carried by a plant transformation vector, which plant transformation vector is an *Agrobacterium tumefaciens* vector.

9. A plant cell containing a DNA construct according to claim 1.

10. A transgenic plant comprising plant cells according to claim 9.

11. A method of making a transgenic plant cell having increased resistance to fumonosin, said method comprising:

providing a plant cell;

providing an exogenous DNA construct, which construct comprises, in the 5' to 3' direction, a promoter operable in a plant cell and a DNA sequence that encodes an ATP-binding cassette transporter protein that increases resistance of a plant cell to fumonosin, said DNA sequence operably associated with said promoter; and

transforming said plant cell with said DNA construct to produce a transformed plant cell, said plant cell having increased resistance to fumonosin compared to an untransformed cell.

12. The method of claim 11, wherein said plant cell is a corn cell.

13. The method of claim 11, further comprising regenerating a plant from said transformed plant cell.

14. A method according to claim 11, wherein said promoter is constitutively active.

15. A method according to claim 11, wherein said promoter is selectively active in plant cells.

16. A method according to claim 11, wherein said promoter is selectively active in plant cells selected from root cells, seed cells, seed coat cells, and corn silk cells.

17. A method according to claim 11, wherein said promoter is a corn Pedicel Glutamine Synthetase promoter that is expressed in the region of the corn kernel attached to the cob.

18. A method according to claim 11, wherein said transforming step is carried out by bombarding said plant cell with microparticles carrying said DNA construct.

19. A method according to claim 11 wherein said transforming step is carried out by infecting said plant cell with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying said DNA construct.

20. A method of producing transgenic plant seeds, comprising collecting seed from a transgenic plant produced by the method of claim 11.

21. The method according to claim 11, wherein said exogenous DNA sequence comprises a DNA sequence selected from the DNA sequences of Claim 1.

22. A transgenic plant having increased resistance to fumonosin compared to a non-transformed control plant, said transgenic plant comprising transgenic plant cells containing:

an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in said plant cell and a DNA sequence that encodes an ATP-binding cassette transporter protein that increases resistance of a plant cell to fumonosin, said DNA sequence operably associated with said promoter;

said plant exhibiting increased fumonosin resistance compared to a non-transformed control plant.

23. The method according to claim 22, wherein said exogenous DNA sequence comprises a DNA sequence selected from the DNA sequences of claim 1.

24. A plant according to claim 22, wherein said promoter is a constitutively active promoter.

25. A plant according to claim 22, wherein said promoter is selectively active in plant cells.

26. A method according to claim 22, wherein said promoter is selectively active in plant cells selected from root cells, seed cells, seed coat cells, and corn silk cells.

27. A method according to claim 22, wherein said promoter is a corn Pedicel Glutamine Synthetase promoter that is expressed in the region of the corn kernel attached to the cob.

28. A transgenic plant according to claim 22, which plant is corn.

29. A transgenic corn plant having increased fumonosin resistance compared to a non-transformed control plant, wherein said transgenic plant is a progeny of a plant according to claim 28.

30. Seeds of a transgenic corn plant having increased fumonosin resistance relative to a non-transformed control plant, wherein said transgenic plant is a plant according to claim 28 or a progeny thereof.

31. A method of producing transgenic plant seeds, comprising collecting seed from a transgenic plant produced by the method of claim 22.

SEQUENCE LISTING

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Boss, Wendy F.
Mao, Cungui

<120> Fumonosin Resistance Proteins

<130> Obeid et al.

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Ile Lys Tyr Tyr Thr Trp Glu Asp Ala Tyr Glu Lys Asn Ile Gln Asp
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Ile Arg Thr Lys Glu Ile Ser Lys Val Arg Lys Met Gln Leu Ser Arg
420 425 430

Asn Phe Leu Ile Ala Met Ala Met Ser Leu Pro Ser Ile Ala Ser Leu
435 440 445

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Val Thr Phe Leu Ala Met Tyr Lys Val Asn Lys Gly Gly Arg Gln Pro
    450                 455                 460

Gly Asn Ile Phe Ala Ser Leu Ser Leu Phe Gln Val Leu Ser Leu Gln
465                 470                 475                 480

Met Phe Phe Leu Pro Ile Ala Ile Gly Thr Gly Ile Asp Met Ile Ile
                485                 490                 495

Gly Leu Gly Arg Leu Gln Ser Leu Leu Glu Ala Pro Glu Asp Asp Pro
            500                 505                 510

Asn Gln Met Ile Glu Met Lys Pro Ser Pro Gly Phe Asp Pro Lys Leu
        515                 520                 525

Ala Leu Lys Met Thr His Cys Ser Phe Glu Trp Glu Asp Tyr Glu Leu
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Asn Asp Ala Ile Glu Glu Ala Lys Gly Glu Ala Lys Asp Glu Gly Lys
545                 550                 555                 560

Lys Asn Lys Lys Lys Arg Lys Asp Thr Trp Gly Lys Pro Ser Ala Ser
                565                 570                 575

Thr Asn Lys Ala Lys Arg Leu Asp Asn Met Leu Lys Asp Arg Asp Gly
            580                 585                 590

Pro Glu Asp Leu Glu Lys Thr Ser Phe Arg Gly Phe Lys Asp Leu Asn
        595                 600                 605
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Phe Asp Ile Lys Lys Gly Glu Phe Ile Met Ile Thr Gly Pro Ile Gly
610 615 620

Thr Gly Lys Ser Ser Leu Leu Asn Ala Met Ala Gly Ser Met Arg Lys
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Thr Asp Gly Lys Val Glu Val Asn Gly Asp Leu Leu Met Cys Gly Tyr
645 650 655

Pro Trp Ile Gln Asn Ala Ser Val Arg Asp Asn Ile Ile Phe Gly Ser
660 665 670

Pro Phe Asn Lys Glu Lys Tyr Asp Glu Val Val Arg Val Cys Ser Leu
675 680 685

Lys Ala Asp Leu Asp Ile Leu Pro Ala Gly Asp Met Thr Glu Ile Gly
690 695 700

Glu Arg Gly Ile Thr Leu Ser Gly Gly Gln Lys Ala Arg Ile Asn Leu
705 710 715 720

Ala Arg Ser Val Tyr Lys Lys Lys Asp Ile Tyr Leu Phe Asp Asp Val
725 730 735

Leu Ser Ala Val Asp Ser Arg Val Gly Lys His Ile Met Asp Glu Cys
740 745 750

Leu Thr Gly Met Leu Ala Asn Lys Thr Arg Ile Leu Ala Thr His Gln

755 760 765

Leu Ser Leu Ile Glu Arg Ala Ser Arg Val Ile Val Leu Gly Thr Asp
770 775 780

Gly Gln Val Asp Ile Gly Thr Val Asp Glu Leu Lys Ala Arg Asn Gln
785 790 795 800

Thr Leu Ile Asn Leu Leu Gln Phe Ser Ser Gln Asn Ser Glu Lys Glu
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Asp Glu Glu Gln Glu Ala Val Val Ala Gly Glu Leu Gly Gln Leu Lys
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Tyr Glu Ser Glu Val Lys Glu Leu Thr Glu Leu Lys Lys Lys Ala Thr
835 840 845

Glu Met Ser Gln Thr Ala Asn Ser Gly Lys Ile Val Ala Asp Gly His
850 855 860

Thr Ser Ser Lys Glu Glu Arg Ala Val Asn Ser Ile Ser Leu Lys Ile
865 870 875 880

Tyr Arg Glu Tyr Ile Lys Ala Ala Val Gly Lys Trp Gly Phe Ile Ala
885 890 895

Leu Pro Leu Tyr Ala Ile Leu Val Val Gly Thr Thr Phe Cys Ser Leu
900 905 910

Phe Ser Ser Val Trp Leu Ser Tyr Trp Thr Glu Asn Lys Phe Lys Asn
915 920 925

Arg Pro Pro Ser Phe Tyr Met Gly Leu Tyr Ser Phe Phe Val Phe Ala
930 935 940

Ala Phe Ile Phe Met Asn Gly Gln Phe Thr Ile Leu Cys Ala Met Gly
945 950 955 960

Ile Met Ala Ser Lys Trp Leu Asn Leu Arg Ala Val Lys Arg Ile Leu
965 970 975

His Thr Pro Met Ser Tyr Ile Asp Thr Thr Pro Leu Gly Arg Ile Leu
980 985 990

Asn Arg Phe Thr Lys Asp Thr Asp Ser Leu Asp Asn Glu Leu Thr Glu
995 1000 1005

Ser Leu Arg Leu Met Thr Ser Gln Phe Ala Asn Ile Val Gly Val Cys
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Val Met Cys Ile Val Tyr Leu Pro Trp Phe Ala Ile Ala Ile Pro Phe
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Leu Leu Val Ile Phe Val Leu Ile Ala Asp His Tyr Gln Ser Ser Gly
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Arg Glu Ile Lys Arg Leu Glu Ala Val Gln Arg Ser Phe Val Tyr Asn
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Ser Gln Glu Arg Phe Leu Ala Lys Ser Asp Phe Leu Ile Asn Lys Met
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Asn Glu Ala Gly Tyr Leu Val Val Val Leu Gln Arg Trp Val Gly Ile
105 1110 1115 1120

Phe Leu Asp Met Val Ala Ile Ala Phe Ala Leu Ile Ile Thr Leu Leu
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Cys Val Thr Arg Ala Phe Pro Ile Ser Ala Ala Ser Val Gly Val Leu
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Leu Thr Tyr Val Leu Gln Leu Pro Gly Leu Leu Asn Thr Ile Leu Arg
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Ala Met Thr Gln Thr Glu Asn Asp Met Asn Ser Ala Glu Arg Leu Val
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Thr Tyr Ala Thr Glu Leu Pro Leu Glu Ala Ser Tyr Arg Lys Pro Glu
185 1190 1195 1200

Met Thr Pro Pro Glu Ser Trp Pro Ser Met Gly Glu Ile Ile Phe Glu
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1220 1225 1230

Leu Asn Leu Asn Ile Lys Ser Gly Glu Lys Ile Gly Ile Cys Gly Arg
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Glu Leu Thr Ala Gly Lys Ile Leu Ile Asp Asn Val Asp Ile Ser Gln
265 1270 1275 1280

Leu Gly Leu Phe Asp Leu Arg Arg Lys Leu Ala Ile Ile Pro Gln Asp
1285 1290 1295

Pro Val Leu Phe Arg Gly Thr Ile Arg Lys Asn Leu Asp Pro Phe Asn
1300 1305 1310

Glu Arg Thr Asp Asp Glu Leu Trp Asp Ala Leu Val Arg Gly Gly Ala
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Ile Ala Lys Asp Asp Leu Pro Glu Val Lys Leu Gln Lys Pro Asp Glu
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Asn Gly Thr His Gly Lys Met His Lys Phe His Leu Asp Gln Ala Val
345 1350 1355 1360

Glu Glu Glu Gly Ser Asn Phe Ser Leu Gly Glu Arg Gln Leu Leu Ala
1365 1370 1375

Leu Thr Arg Ala Leu Val Arg Gln Ser Lys Ile Leu Ile Leu Asp Glu
1380 1385 1390

Ala Thr Ser Ser Val Asp Tyr Glu Thr Asp Gly Lys Ile Gln Thr Arg
1395 1400 1405

Ile Val Glu Glu Phe Gly Asp Cys Thr Ile Leu Cys Ile Ala His Arg
1410 1415 1420

Leu Lys Thr Ile Val Asn Tyr Asp Arg Ile Leu Val Leu Glu Lys Gly
425 1430 1435 1440

Glu Val Ala Glu Phe Asp Thr Pro Trp Thr Leu Phe Ser Gln Glu Asp
1445 1450 1455

Ser Ile Phe Arg Ser Met Cys Ser Arg Ser Gly Ile Val Glu Asn Asp
1460 1465 1470

Phe Glu Asn Arg Ser
1475

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 98/17546

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/31 A01H5/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LU, Y.-P., ET AL.: "AtMRP1 gene of Arabidopsis encodes a glutathione S-conjugate pump: Isolation and functional definition of a plant ATP-binding cassette transporter gene" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 94, 22 July 1997, pages 8243-8248, XP002083621 WASHINGTON US see the whole document	1
P,X	WO 98 21938 A (UNIV PENNSYLVANIA) 28 May 1998 see page 90 - page 91; claims 1-26; figures 13-18	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 1998	24/11/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

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Int Ional Application No
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A	VOET, M.., ET AL.: "S. cerevisiae chromosome VII reading frame ORF YGR281w" EMBL SEQUENCE ACCESSION NO. Z73066, 17 May 1996, XP002083622	1
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